

Kinetic Modeling of Metabolic Pathways in *Zymomonas mobilis* to Optimize Pentose Fermentation

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Zymomonas mobilis has been engineered with four new enzymes to ferment xylose along with glucose. A network of pentose pathway enzymatic reactions interacting with the native glycolytic Entner Doudoroff pathway has been hypothesized. We have analyzed the complex interactions between the pentose phosphate and glycolytic pathways in this network by developing a large-scale kinetic model for all the enzymatic reactions. Based on the experimental literature on *in vitro* characterization of each of the 20 enzymatic reactions, the large-scale kinetic model is numerically simulated to predict the dynamics of all the intracellular metabolites along the network of interacting metabolic pathways. This kinetic model takes into account all the feedback and allosteric regulations on the enzymatic reaction rates and is better suited to the systems level analysis of interacting metabolic pathways compared to the standard linearized methods of meta-bolic flux analysis and metabolic control theory.

This nonlinear kinetic model is simulated to perform numerous *in silico* experiments by varying different enzyme concentrations and predicting their effects on the intracellular metabolic concentrations and the ethanol production rates in continuous fermentors. Among the five enzymes whose concentrations were varied and given as input to the model, the ethanol concentration in the continuous fermentor was optimized with xylosisomerase was needed at the highest level, followed by the transaldolase. Predictions of the model, that inter-connecting enzyme phosphoglucose isomerase, does not need to be over expressed, were recently confirmed through experimental investigations. Through this kinetic modeling approach, we can develop efficient ways of maximizing the fermentation of both glucose and xylose, while minimizing the expression of the heterologous enzymes.